IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Sackstein		
SERIAL NUMBER:	10/042,421	EXAMINER:	Phillip Gambel
FILING DATE:	October 18, 2001	ART UNIT:	1644
TITLE:	HEMATOPOIETIC CELL E-SELECTIN/ L-SELECTIN LIGAND POLYPEPTIDES AND METHODS OF USE THEREOF		

Mail Stop Amendment
Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF LOUIS PICKER UNDER 37 C.F.R. § 1.132

I, Louis Picker, declare and state:

1. I am a Professor at Oregon Health Sciences University. I received my M.D. from University of California, San Francisco, in 1982. I completed residency training in anatomic pathology and subsequently was a post-doctoral research fellow in the laboratory of Dr. Eugene Butcher at Stanford University from 1987-1990. In that time, I found that monoclonal antibody HECA-452 reacted against a carbohydrate determinant that conferred E-selectin binding activity. I have been working in the field of immunology for over 30 years. I am an expert on selectin ligands, having been the first person to define E-selectin ligands of hematopoietic cells, and am world-recognized for my contributions regarding the glycobiology of selectin ligands.

2. I have reviewed the Office Action dated May 21, 2008 and the specification of the present application. I understand the content and scope of claims 1-4, and 7 pending in the above-captioned application; the claims are directed to highly purified preparations of glycosylated polypeptides comprising a CD44 amino acid backbone and sialylated, fucosylated glycans and having E-selectin and/or L-selectin ligand activity. I understand that the examiner questions the novelty and inventiveness of the pending claims. I

understand that the rejections set forth by the examiner are based, *inter alia*, on the publications of Sackstein 1997,[1/] Sackstein 2004[2], Dougherty[3/], and Stamenkovic[4/].

3. I make this declaration to rebut the Examiner's rejection, with which I do not agree.

4. I am fully aware of the history of the discovery of HCELL. The identification of this molecule is tied to the development of the "blot rolling assay", which was first described in the present application. For this work, Robert Sackstein, the inventor of the present application, is credited by myself and other fellow colleagues as having identified a novel class of CD44 glycoproteins, referred to as HCELLs.

5. No other reports prior to this date provided information that would have led one of skill in the art to the notion that HCELL was a CD44 glycoprotein. Specifically, Sackstein 1997[5/] teaches of the existence of an L-selectin ligand expressed on the human hematopoietic cell line KG1a that is structurally distinct from all other previously identified L-selectin ligands by virtue of displaying sulfation-independent binding activity. There is no mention of the identity of the putative ligand in this or in any other published work by others, only that its activity as a selectin ligand does not require sulfation. Many molecules lack sulfation, and thus the identity of the polypeptide backbone could not be determined by this property. Notably, CD44 is known to be sulfated, which would have led anyone knowledgeable in the field to exclude the role of CD44 as a selectin ligand.

1/ Sackstein R, Fu L, Allen KL; "A hematopoietic cell L-selectin ligand exhibits sulfate-independent binding Activity;" Blood. 1997 Apr 15;89(8):2773-81.

2/ Sackstein R.; "The bone marrow is akin to skin: HCELL and the biology of hematopoietic stem cell Homing;" J Invest Dermatol. 2004 May;122(5):1061-9. Review.

3/ Dougherty GJ, Landorp PM, Cooper DL, Humphries RK.; "Molecular cloning of CD44R1 and CD44R2, two novel isoforms of the human CD44 lymphocyte 'homing' receptor expressed by hemopoietic cells;" J Exp Med. 1991 Jul 1;174(1):1-5.

4/ Stamenkovic I, Aruffo A, Amiot M, Seed B.; "The hematopoietic and epithelial forms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate-bearing cells;" EMBO J. 1991 Feb;10(2):343-8.

5/ Sackstein R, Fu L, Allen KL; "A hematopoietic cell L-selectin ligand exhibits sulfate-independent binding Activity;" Blood. 1997 Apr 15;89(8):2773-81.

6. Indeed, my own studies in the early 1990s would teach that native CD44 immunoprecipitated from hematopoietic cells would not be a selectin ligand. Importantly, I (personally) immunoprecipitated CD44 and found, specifically, that it lacked HECA452 reactivity,[6/] and, indeed, that CD44 immunoprecipitated from a human hematopoietic cell type (lymphocytes from human tonsil) did not support binding of E-selectin-expressing cells (ELAM-1-transfected cells), whereas protein(s) isolated by using HECA-452 mAb did support binding of E-selectin-expressing cells.[7/] That is, isolated CD44 was used to show that the comparator HECA-452 immunoprecipitated protein(s) possessed E-selectin binding activity, but that CD44 did not possess such E-selectin binding activity. In this manner, isolated, purified CD44 was frequently used as a "negative control" by us and others to compare against authentic selectin ligands precisely because it was shown to be non-reactive with HECA-452 mAb and found to be devoid of selectin ligand activity. As such, no one would have thought that a sialofucosylated glycoform of CD44 existed that would function as a selectin ligand, i.e., no one would have ever predicted that HCELL would be a CD44 glycoprotein.

7. In contrast, the present specification teaches a highly purified (e.g., isolated) preparation of a glycosylated form of CD44 that is an E-selectin ligand (i.e., HCELL). This glycosylated form of CD44 is reactive with monoclonal antibody HECA-452. HECA-452 recognizes sialofucosylated glycans. The presence of these sialofucosylated glycans confers L-selectin and E-selectin binding. It would not be sufficient to isolate the HCELL glycoform of CD44 using antibodies directed solely to CD44, as the critical combination of HECA-452-reactivity and anti-CD44 antibody-reactivity, used together, would be necessary to isolate this species.

8. I have also reviewed Sackstein 2004 and rebut the Examiner's assertion that Sackstein 2004 provides evidence that the claims are not novel. Specifically, I rebut the Examiners assertion that the pending claims lack novelty because of the following statement made in Sackstein 2004:

[6/] Picker *et al.*, A Unique Phenotype of Skin-associated Lymphocytes in Humans, American Journal of Pathology, 136:1053-1068, 1990.

[7/] Berg *et al.*, The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1, J Exp Med. 1991;174:1461-1466.

Although initially considered to be a novel selectin ligand by the above biochemical criteria, mass spectrometry subsequently revealed that HCELL is not novel *per se*: it is a glycoform of a well-recognized intergral membrane glycoprotein, CD44, that expresses the CLA epitope (i.e., is recognized by mAb HECA-452).

9. The statement above merely acknowledges that the identity of the backbone was a known polypeptide rather than some previously undiscovered polypeptide backbone. The statement does not mean that the unique glycoprotein with a CD44 polypeptide backbone having a particular sialofucosylated carbohydrate structure that binds E-selection or L-selection and is reactive with monoclonal antibody HECA-452 is not novel.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001.

Louis Picker

Aug 5, 2007
Date

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